

# **Exhibit 8**

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Book No. \_\_\_\_\_ 02/18/2016

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Aegeline Study

Aegeline received from Dr. Amar / Dr. Saglain.

02/19/2016

Received caffeine 500mg from Dr. Amar / Saglain.

MTD Study :- Aegeline :-

Animals used :- Male ND4 mice.

Mice #1 :- 21.30 9:03am I.L. Aegeline 500mg/kg

Mice #2 :- 21.92g 9:30 am I.L. Aegeline 1000mg/kg

Observation :-

- 1 - 2 hours :- Mice activity was increased
- - 3 hours :- Mice activity back to normal
- 6 hours :- Normal activity.

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Test :- Caffeine and Aegeline.

→ One mouse given caffeine (50mg/kg b.wt) I.E at 10:13 am.

→ Then at 10:28 am given aegeline 500mg/kg I.E.

Observation :-

After 15 minutes of aegeline dose, mouse activity was decreased, hair straight, breath fast lasted for 2 hrs.

After 4 hours, Mouse recovered to normal.

Solution Preparation :-

Caffeine :- 6.25 mg caffeine dissolved in 1250  $\mu$ l (1.25 ml) of  $H_2O$ .  
equivalent to 5mg/ml.

Aegeline :- 100mg + gum acacia (10mg)

↓  
Pestle mortar

↓  
made up to 1.0ml by D.D  $H_2O$ .

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## Pharmacokinetic (PK) study of Aegeline

Animal :- Male ND4 mice ✓

:- 14 Nos.

Aegeline dose :- 30mg/kg

2 mice as Control given vehicle ( $H_2O$  + gum acacia)

12 mice given 30mg/kg of Aegeline I.e.

→ Two mice were sacrificed at 30 min, 1 hour, 2 hrs, 4 hr, 6 hr and 8 hrs in time intervals after aegeline administration

→ Blood samples were collected at these time points by cardiac puncture in EDTA-coated tubes (pink top).

→ Plasma was separated by centrifuging blood samples at 4000 rpm for 5 min  $20^{\circ}C$ .

→ Tissue samples: liver, kidney and brain were also taken and stored at  $-80^{\circ}C$ .

Plasma and tissue samples given to Dr. Shabana/Dr. Vamshee for analysis.

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Mouse #		Weight (g)	Doug given Time	Blood Collected Time	Time point
1.	1.	18.29	9:00	9:30 am	30 mins
1.	2.	22.77			
3.		20.53	9:10	10:10 am	1 hour
4.		23.17			
5.		24.4	9:12	11:12 am	2 hours
6.		24.62			
7.		21.44	10:00 am	2:00 pm	4 hours
8.		22.06			
9.		22.57	9:15 am	3:15 pm	6 hours
10.		22.13			
11.		20.75	8:06 am	4:06 pm	8 hours
12.		19.58			
13.		24.65	CONTROL		0 hours
14.		23.34			



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Organ weights (g) Aezeline 30mg/kg Pk. Study Mice

	Mouse #	Liver	Brain	Kidney
30 min	1.	0.72	0.34	0.28
	2.	0.93	0.37	0.36
1 hour	3.	0.80	0.29	0.36
	4.	0.97	0.38	0.30
2 hours	5.	1.17	0.39	0.36
	6.	0.89	0.32	0.32
4 hours	7.	1.08	0.39	0.28
	8.	0.84	0.39	0.32
6 hours	9.	1.08	0.36	0.42
	10.	0.93	0.42	0.32
8 hours	11.	0.56	0.34	0.30
	12.	0.94	0.34	0.35
0 hour CONTROL	13.	1.84	0.35	0.36
	14.	1.31	0.32	0.38

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From Page No. \_\_\_\_\_ P.K. Study Azelene 300mg/kg mice I.E.

T.	Mouse #	Weight (gm)	Time Drug Given	Time Blood Collection	Time Point
Black.	3	22.30	9:20 am	9:50 am	30 MINS
	4	23.27			
Black	1	23.71	9:19 am	10:19 am	1 HOUR
	2	25.14			
Red	3	22.58	9:12 am	11:12 am	2 HOURS
	4	25.18			
Red	1	25.34	9:08 am	1:08 pm	4 HOURS
	2	26.64			
Green	3	25.09	9:05 am	3:05 pm	6 HOURS
	4	24.95			
Green	1	24.99	9:00 am	5:00 pm	8 HOURS
	2	22.56			

\* 0 HOUR already done in previous study.

Blood and tissue samples were collected in the same way as done before (written on 02/23/16)

Plasma separated and

Tissue stored at  $-80^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$ .

Plasma and tissue samples given to Dr. Shabana/Dr. Renshi for analysis

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Organ Weights (gm) Aeseline 300mg/kg  
 PK. Study mice

Mouse #	Liver	Brain	Kidney
Black 3	0.86	0.30	0.39
4	0.52	0.39	0.31
Black 1	0.75	0.38	0.36
2	0.72	0.35	0.33
Red 3	1.07	0.31	0.29
4	1.08	0.36	0.38
Red 1	1.27	0.29	0.38
2	1.40	0.36	0.47
Green 3	1.29	0.29	0.33
4	1.12	0.29	0.41
Green 1	1.10	0.34	0.34
2	1.06	0.31	0.33



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From Page No. \_\_\_\_\_ P.K Study Aegeline (40mg/kg) in Rats.

Catheterized rats used.

Drug aegeline given 40mg/kg I.e. (500ul/rat)

Time Point	Rat #1	Rat #2	Rat #3	Rat #4
0 Hr.	8:05 <sup>am</sup> ✓	8:07 <sup>am</sup> ✓	8:08 <sup>am</sup> ✓	8:10 <sup>am</sup> ✓
Drug given	8:34 <sup>✓</sup>	8:41 <sup>✓</sup>	8:42 <sup>✓</sup>	8:44 <sup>✓</sup>
30 MIN	9:04 <sup>✓</sup>	9:11 <sup>✓</sup>	9:12 <sup>✓</sup>	9:14 <sup>✓</sup>
1 HR	9:34 <sup>✓</sup>	9:41 <sup>✓</sup>	9:42 <sup>✓</sup>	9:44 <sup>✓</sup>
2 Hr	10:34 <sup>✓</sup>	10:41 <sup>✓</sup>	10:42 <sup>✓</sup>	10:44 <sup>✓</sup>
4 Hr	12:34 <sup>✓</sup>	12:41 <sup>✓</sup>	12:42 <sup>✓</sup>	12:44 <sup>✓</sup>
6 Hr	2:34 <sup>pm</sup> ✗	2:41✗	2:42✗	2:44✗
8 Hr	4:34✗	4:41✗	4:42✗	4:44✗
<del>24hr</del> 7 Hr	3:34 <sup>✓</sup>	3:41 <sup>✓</sup>	3:42 <sup>✓</sup>	3:44 <sup>✓</sup>
31/11/16 24hr.	8:34 <sup>am</sup> ✓	8:41 <sup>✓</sup>	8:42 <sup>✓</sup>	8:44 <sup>✓</sup>

\* X : Blood samples could not be collected at 6hr and 8hr.

Instead 7hr. blood sample were taken.

Plasma separated and given to Dr. Vamsi for analysis.

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1 gram of Aegeline was given by  
Dr. Saglaim / Dr. Arner.

3/15/16

Sub-Acute Toxicity Study for 28 days: - Mic

Group #1 Caffeine 50mg/kg n=7  
03/15/2016  $\xrightarrow{28 \text{ days}}$  04/12/2016  
Sacrifice date  
04/12/16

Group #2 Aegeline 300mg/kg n=7

03/16/2016  $\longrightarrow$  04/13/2016

Group 3 Aegeline 300mg/kg + Caffeine 50mg/kg n=7

03/17/2016  $\longrightarrow$  04/14/2016

Group 4 Control vehicle n=4.

03/18/2016  $\longrightarrow$  04/15/2016

Pooled  
 $\rightarrow$  Urine will be collected after 2 hours of dosing.

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## Body weight (gms) of Aegeline study

Group I	0 day	5 day	10 day	15 day	20 day	28 days
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Group 1:- Caffeine (500mg/kg)	0 day	5 day	10 Day	15 Day	20 <sup>th</sup> Day	28 <sup>th</sup> day
1. 27.04	25.5	26.32	27.34	28.67	28.82	
2. 25.56	22.58	*Died	-	-	2-	
3. 26.38	26.02	25.65	28.11	27.99	28.19	
4. 24.46	21.83	24.30	25.18	25.47	25.46	
5. 29.27	27.23	*Died	-	-	-	
6. 25.21	22.14	*Died	-	-	-	
7. 27.48	25.48	24.02	24.17	24.31	24.68	

## Group 2:- AEGELINE (300mg/kg)

1. 25.24	24.68	24.12	23.51	21.26	22.92
2. 22.68	21.81	22.23	26.07	24.41	24.80
3. 26.34	25.92	26.34	27.32	26.22 <sub>g</sub>	26.46
4. 24.38	24.09	24.14	25.79	25.24	25.14
5. 28.09	27.72	27.08	27.57	29.57	29.42
6. 25.90	24.13	26.45	27.12	27.02	27.64
7. 25.84	25.71	26.67	27.57	27.99	28.54

\* Mechanical error. - mishandling

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Group 3	0 day 5 day		Caffeine (50mg/kg) + Aegeline (300mg/kg)			
	0 Day	5 Day	10 <sup>th</sup> Day	15 <sup>th</sup> Day	Day 20 <sup>th</sup>	28 <sup>th</sup> day
1.	22.66	*Died	-	-	-	
2.	23.03	22.21	22.50	22.93	23.52	24.35
3.	25.09	26.13	27.0	26.80	26.78	27.11
4.	30.62	28.29	29.33	30.48	30.84	32.03
5.	24.88	25.18	25.22	25.34	25.14	25.82
6.	27.06	24.77	24.86	26.30	27.80	26.80
7.	26.85	25.82	26.12	27.76	27.20	27.56

Group 4 (CONTROL GROUP - VEHICLE) ~~§~~.

1.	29.08	29.72	29.76	31.89	31.52	31.89	32.6
2.	23.48	24.81	24.46	26.32	26.01	26.32	
3.	29.10	30.52	30.05	30.80	30.66	30.80	
4.	26.09	26.48	27.33	28.41	28.72	28.41	

28<sup>th</sup> Day

- 1- 32.64
- 2- 26.48
- 3- 31.62
- 4- 28.86

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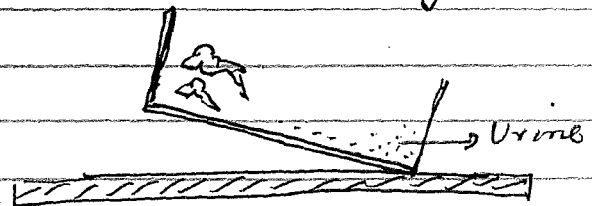
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### \* Urine Collection :

Before urine collection, mice were given 500ul of water 40mins after dosing..

Ur pooled urine was collected.

Mice of one group were kept in clean cage (without bedding) and after dosing and were kept tilted.





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Date: 04/12/2016 to 04/15/2016

Animals were sacrificed after 28 day accordingly.  
 Blood was collected and Liver profile were done  
 on vet scan..

Tissues/organs Liver, kidney, brain and spleen. Collected  
 and weight and stored at  $-20^{\circ}\text{C}$ .

### ORGAN WEIGHTS (gms)

#### Group 1 (Caffeine 50mg/kg)

Mice #	Liver	Kidney	Brain	Spleen
1 -	1.52	0.46	0.45	0.08
3 -	1.46	0.44	0.45	0.09
4 -	1.32	0.42	0.44	0.08
7 -	1.37	0.43	0.39	0.08

#### Group 2 (Aegeline 300mg/kg b. wt)

1 -	1.29	0.33		0.08
2 -	1.21	0.38		0.08
3 -	1.31	0.32		0.06
4 -	1.25	0.36		0.08
5 -	1.62	0.48		0.07
6 -	1.52	0.43		0.09
7 -	1.44	0.40		0.11

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Group 3 (Caffeine 50mg/kg) + Aegeline 300mg/kg

	Liver	Kidney	Spleen
2-	1.34	0.42	0.07
3-	1.46	0.45	0.08
4-	1.57	0.51	0.08
5-	1.43	0.43	0.08
6-	1.44	0.44	0.08
7-	1.37	0.41	0.09

Group 4 (Vehicle Control)

1-	2.01	0.55	0.09
2-	1.55	0.46	0.09
3-	1.87	0.51	0.10
4-	1.67	0.45	0.10
	<u>Liver</u>	<u>Kidney</u>	<u>Spleen</u>

Tissues stored in freezer.

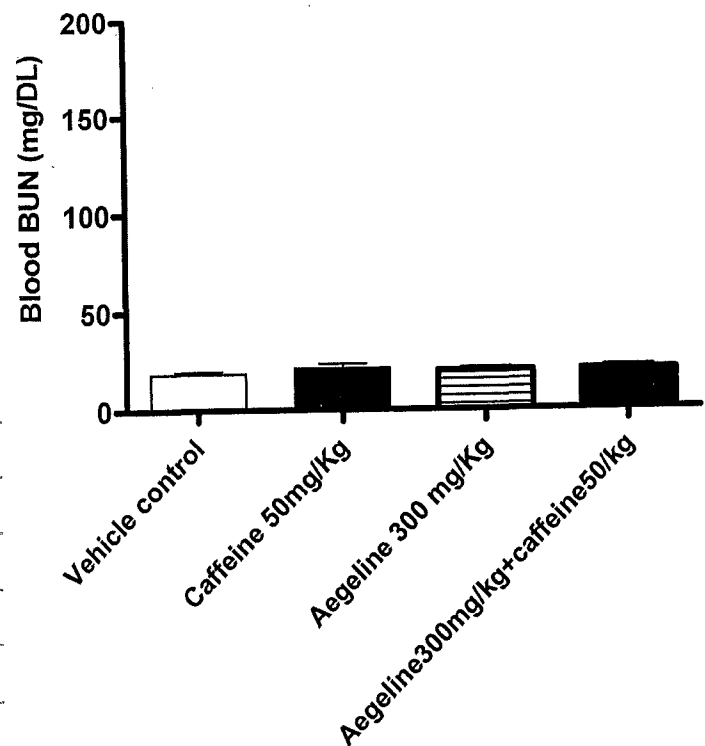
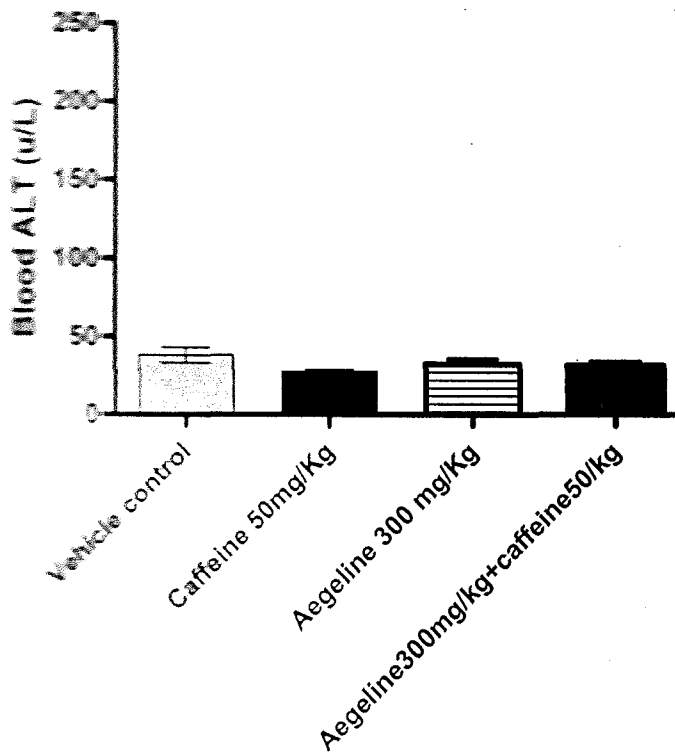
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**Aegeline-caffenige liver toxicity test for 28days- animal survival (3/15/2016-4/14/2016)**

Group	routes	animal no.	death of mice	mortality (%)
G1 (Caffeine 50 mg/Kg once daily)	IG	4	0	0.0
G2 (Aegeline 350 mg/Kg once daily)	IG	7	0	0.0
G3 (Caffeine 50 mg/Kg + Aegeline 350 mg/Kg once daily)	IG	6	0	0.0
G4 (Vehicle control once daily)	IG	4	0	0.0

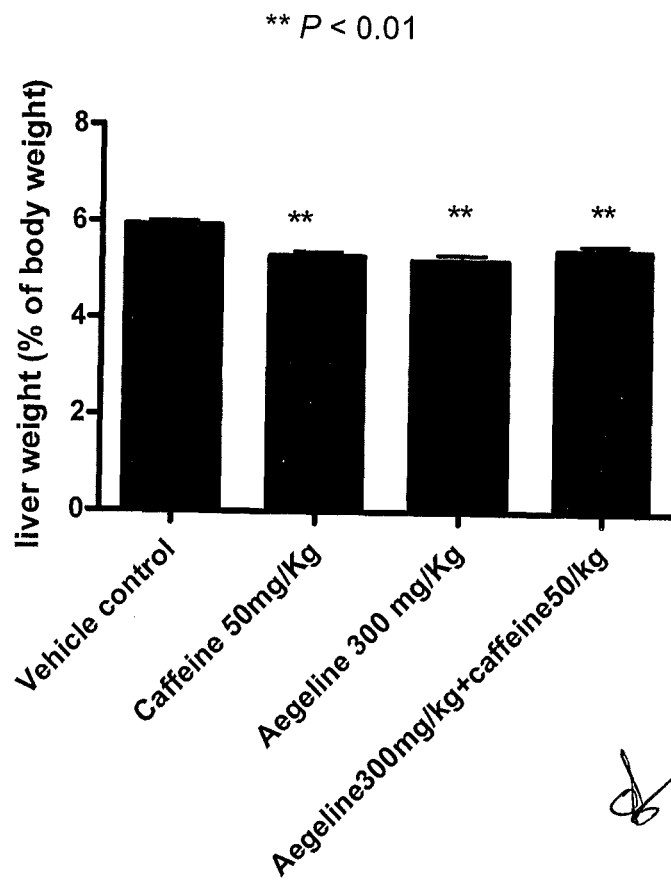


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